

Trace Analysis on Capillary Columns. Selected Practical Applications: Insecticides in Raw Butter Extract; Aroma Head Space from Liquors; Auto Exhaust Gas

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Abstract

Trace analysis on capillary columns becomes feasible provided appropriate injection techniques without stream splitting are used. To demonstrate potentialities and limitations of the methods, we investigated practical applications, the conditions of which were as dissimilar as possible. Insecticides in dairy products can be estimated by direct analysis of the raw petrol ether extract, without previous purification by column chromatography. 2-4 μ l of extract are injected without splitting on a short inlet capillary fitted in a separate injection bloc. The inlet capillary is then connected to the capillary column mounted in the column oven with electron capture detector. Analysis is started by rapid heating to a constant temperature or by temperature programming resp. Head space analysis is another case in which trace components are important. Whereas several workers reported trapping of head space vapors in capillaries at low temperatures, we emphasize avoiding condensation, e.g. of water vapor, in the capillary. All sampling is done, therefore, near the original temperature of the head space material, i.e. at room temperature. In the case of cognac aroma analysis in which most of the water and ethanol vapor are vented through the inlet capillary, the influences of most sampling variables—gas volume, length of inlet capillary—are studied. In the analysis of auto exhaust gas, again, bypassing of water is an important detail. In addition to hydrocarbons originating directly from the fuel as investigated by several authors, we found many combustion and conversion products, primarily naphthalene derivatives and phenols.

Introduction

It has long been common opinion that the special ability of capillary columns was high separation efficiency, whereas, due to their geometrical structure, analyses of large and highly diluted samples were out of their competence. In a previous paper (1) we studied the concentration effect occurring, when during injection the temperature of the column is held at least ca. 50° below normal analysis temperature. We showed that to obtain concentration on the column with optimum result of the subsequent analysis, it is not necessary to freeze the injected sample in a small portion of the column.

The concentration effect allows the sample size and, in parallel, the injection time, to be drastically increased without loss of separation. In addition to making stream splitting dispensable, this allows the

corresponding drastic increase of sample dilution thus bringing about the conditions of trace analysis.

To check the applicability of our methods we selected analytical problems with completely different sample types. Not being experts in either of the corresponding specialized areas we wish to emphasize that we present our procedures and results without trying to compare their practical value with that of actually used methods.

General Considerations

Liquid sample size; handling of solvent

In contrast to the concentration conditions with freezing we adjust the injection conditions in such a way that the solvent is not strongly retained. Thus even large amounts of solvents do not harm the column since no plugging of the capillary at the concentration point is possible and, furthermore, no rapid heating of the frozen sample with corresponding formation of a very concentrated zone of gaseous solvent is needed.

Therefore, the sample size for direct injection is only limited by the need of resolving the most volatile sample components from the solvent. Excessive amounts of solvent will be eluted as very broad peaks obscuring the earlier sample peaks. A less often observed limitation may derive from special detectors such as the ECD which may be heavily disequibrated by large amounts of solvent vapors.

A substantial increase of sample size beyond these limits is practicable using the solvent bypassing technique. If there is sufficient volatility difference between the solvent and the most volatile sample component the solvent can be bypassed completely. In the other cases the optimum compromise must be chosen. As a rough rule applying to capillaries with 3-35 mm, i.d., we use direct splitless injection up to 2-3 μ l and solvent bypassing for larger sample sizes.

Inlet capillaries for solvent bypassing

We were interested to know within what limits the inlet capillary has to be of identical dimensions and

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1. Grob, K., and Grob, G., J. Chromatog. Sci. 7, 587 (1969).

coating as the main column. The results were the following:

To avoid losses in separation efficiency the i.d. as well as the thickness of the liquid film should remain within ca. $\pm 10\%$ of the figures for the main column. On the other hand, the choice of the liquid phase is completely free, i.e. we did not observe any significant change in efficiency when, instead of identical coatings, we combined a nonpolar column with an inlet capillary coated with a liquid phase of high polarity and vice versa. To facilitate solvent bypassing the polarity of the inlet capillary should, as a rule, differ as much as possible from that of the solvent.

Gaseous samples

Trace components in gaseous mixtures have been concentrated on capillaries by several authors (2-6). Their normal procedure was to cool a short piece of capillary with dry ice or liquid nitrogen. The sample size becomes strongly limited especially when large amounts of condensable vapors such as water are present. To avoid plugging of the capillary it would seem helpful to raise the concentration temperature to a point where condensation without freezing occurs. With glass capillaries it is easy to observe that every condensed droplet having attained a critical size starts moving with the flowing gas and destroys the coating film along its way while, at the former position, a new drop is built up. A similar effect is normally observed even with freezing. Upon heating the cooled section, the condensed material, just after melting, is blown away before being vaporized.

The only way to overcome this trouble is to avoid condensation. For example, with gases almost saturated with water or ethanol, this means that during sampling, the capillary must be held at the temperature of the gas mixture. The concentration capacity of the method is limited by the possible loss of volatile material being vented at the end of the inlet capillary. In many instances partial venting, e.g. of water vapor, is desired. To some extent this bypassing of sample components can be made selective by properly choosing the polarity of the coating for the inlet capillary.

If the concentration effect is still insufficient, further concentration of trace substances can be accomplished by using the method described by Janak and coworkers (7) based on frontal analysis. We shall discuss the application of Janak's technique in a separate paper.

Applications with Liquid Injection

"Semi-Volatiles" of tobacco smoke

In this instance a very dilute solution, the bulk of which is volatile, must be analyzed. The relatively high dilution derives from the need of dissolving antagonistic substances, such as long chain paraffins with glycols, in the same solvent system and from the danger of secondary reactions between reactive smoke components. Up to $5 \mu\text{l}$ of smoke solution were injected without splitting on an inlet capillary using solvent bypassing. This work has been described separately (8,9).

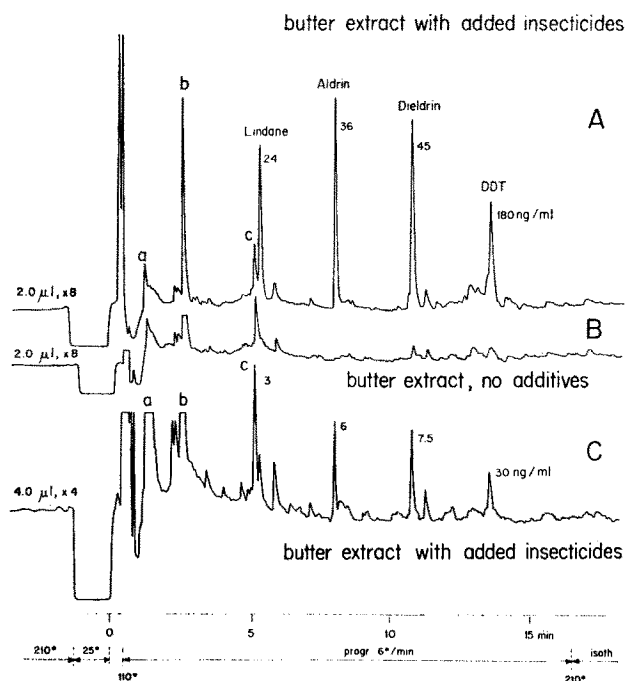


Figure 1. B: raw hexane extract from "insecticide free" butter; A and C: same extract with added insecticides. Glass capillary, 20 m/0.32 mm, silicone oil SF 96. Carrier hydrogen, 0.6 atmos. (3.5 ml/min). App.: Carlo Erba, Mod. GI, ECD, ^{63}Ni , 3.5 V DC, 350° , 65 ml nitrogen/min. Atten. 100 x 8 and 100 x 4 resp.

Insecticides in raw butter extract

Here, relatively volatile trace substances dissolved in a large amount of butter fat must be vaporized and resolved. The determination of insecticides in dairy products actually used involves a laborious purification of the hexane extract. A drastic simplification is conceivable provided two conditions are fulfilled: 1.) the insecticides are quantitatively evaporated out of the raw extract without interfering evaporation of fat or other butter constituents; and 2.) the insecticides are effectively concentrated on the capillary column.

The chromatograms in Figure 1 are obtained in the following manner: a 1 m piece of glass capillary coated with silicone oil SF 96 is inserted, with most of its

- Heins, J. T., Maarse, H., ten Noever de Brauw, M. C., and Weurman, C., *J. Gas Chromatog.* 4, 395 (1966).
- Weurman, C., *Proc. 2nd Internat. Congr. Food Sci. Technol.* 298 (1966).
- Ten Noever de Brauw, M. C., and Brunnée, C., *Z. Anal. Chem.* 5, 321 (1967).
- Ten Noever de Brauw, M. C., and Schuy, K. D., *Proc. Internat. Symp. Chromato-Mass Spectrometry, Moscow*, 214 (1968).
- Grob, K., and Grob, G., *J. Chromatog. Sci.* 7, 584 (1969).
- Novak, J., Vasak, V., and Janak, J., *Anal. Chem.* 37, 660 (1965).
- Grob, K., and Voellmin, J. A., *Beitr. Tabakforsch.* 5, 52 (1969).
- Grob, K., and Voellmin, J. A., *J. Chromatog. Sci.* 8, 218 (1970).

length at room temperature, in a separate vaporizer block designed as shown by Figure 1., p. 585 in a previous paper (6). The block has the same carrier gas supply as the vaporizer in a regular gas chromatograph but has no splitting device. A loose plug of glass wool in the vaporizer tube protects the capillary entrance from contact with sample droplets produced by rapid injection.

The injection procedure is described on p. 589 (1). The exact conditions are: flow through inlet capillary 2 ml nitrogen/min, inlet pressure .05 at block temperature 210°. 60 seconds after rapid injection (to provide wide distribution of non volatile material) of 2 μ l, the inlet capillary is withdrawn from the block. After connecting its outlet end to the main column, the inlet end is fitted into the carrier gas supply. As soon as the air is eluted, i.e. when the detector indicates elution of solvent, the column oven is closed and rapidly heated to 110°. From 110-210° the temperature is raised 6°/min. Meanwhile the glass tube, heavily loaded with butter fat, is removed from the vaporizer block for cleaning. A clean tube is inserted and conditioned for the next run.

We used a hexane extract with 100 mg fat per ml from an "insecticide free" alp butter (chromatogram B). The majority of the peaks, especially a,b,c, are not due to vaporized butter material but to septum bleed. A and C show chromatograms from the same extract with added insecticides whereby C is run with double sample size and half attenuation.

Table I shows the influence of the evaporation conditions (temperature and duration) on the peak height for dieldrin obtained with a butter extract containing 40 ng of dieldrin per ml. It is seen that under our selected conditions, 210° and 60 sec, the insecticide is almost fully evaporated. Further increases of both temperature and duration are undesirable because they would increase septum bleed and contamination of the column by fatty material. Under these conditions dispersion of the results was $\pm 3\%$ mainly due to fluctuations of the sample size. Injections were made with "solvent flushing" (10).

To judge the recovery of insecticides from butter fat we added equal amounts of dieldrin to butter extract and to pure hexane respectively. In repeated runs using several independent "insecticide free" extracts as well as different solvents for the pure solution we obtained 10-15% higher dieldrin figures from butter extracts, i.e. the contrary of what we had expected.

The minimum detectable dieldrin concentration was in the order of 1 ng/ml. We believe that using a vaporizer design with reduction of septum bleed we shall be able to detect substantially lower concentrations.

Applications with Gaseous Sampling

Aroma head space from cognac

The specific problem of this kind of analysis is the following: traces of aromatic substances are to be estimated in a gas mixture in which water and ethanol are by far predominant.

In the case of concentration by freezing the limitation of the sample size is the main difficulty. With our procedure of splitless injection larger samples can be

Table I. Recovery of dieldrin from butter fat under different evaporation conditions.

	Temperature of vaporizer block (°C.)				Peak height for dieldrin (mV $\cdot 10^{-2}$)
	180	210	220		
Time interval between in-	20	36	64	78	
jection and	40	48	82	83	
removal of	60	60	84	85	
inlet capillary.	80	67	85	84	
(seconds)					

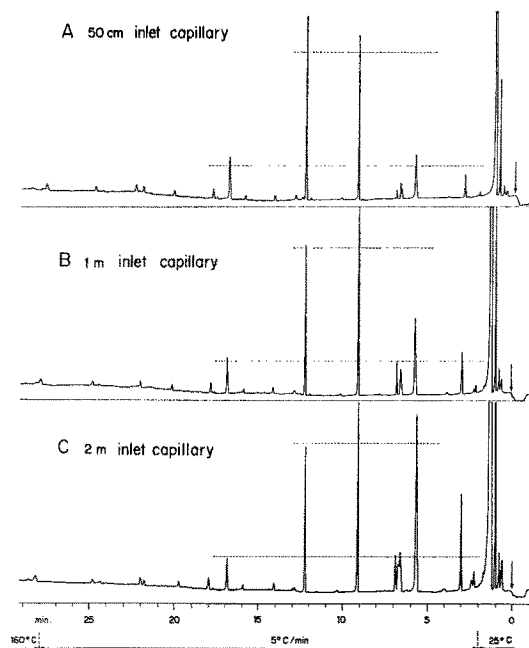


Figure 2. Cognac aroma head space. 4 ml of head space gas introduced into inlet capillaries of different length. Glass capillary, 25 m/0.35 mm, Emulphor O alkaline. Carrier Hydrogen, 0.3 atmos. (2.5 ml/min). App.: Carlo Erba, Mod. GI, FID.

handled. However, a very broad ethanol peak is obtained which tends to trouble the earlier part of the chromatogram. With solvent bypassing using a non-polar inlet capillary the bulk of water and ethanol can be vented. In this case, instead of being covered by the ethanol peak, the same substances suffer a loss, the extent of which is a function of their volatility.

Our sampling procedure was the following: the cylindric 250 ml flask containing 100 ml of cognac and covered with aluminum foil was held at room temperature (22°); a separate inlet capillary was inserted through the foil down to a distance of a few mm over the liquid; and the opposite end was connected to the water pump via a flow restrictor yielding a flow of 2 ml/min through the capillary. We used this example to study two main sampling variables.

1. Influence of length of inlet capillary: The results are shown by Figure 2. Since the sample size was

constant (4 ml, corresponding to 2 min of suction) we expected identical chromatograms for the less volatile substances. We observed, however, a slight reduction of peak heights with increasing length of inlet capillary. The effect is best demonstrated by the peaks eluted at 13 and 17 minutes respectively. The reduction is reproducible and affects all substances in the second half of the chromatograms. At present we have no satisfactory explanation for it.

The earlier substances, being partially vented, show the expected increase with increased inlet length. The heights of the peaks at 3 and 6 min respectively, are almost proportional to the inlet length. For these substances we were prepared to obtain, from the long inlet, broadened or distorted peaks because of the distribution of the respective substances over a capillary length corresponding to almost 10 seconds of carrier flow. This phenomenon was not detectable.

2. Influence of sample size: Sample volumes of 2, 4, and 8 ml as obtained by suction during 1, 2, and 4 min were introduced into the same inlet capillary of 1 m length. Since the variation of sample size was compensated by proportionally varied attenuation, identical chromatograms were to be expected with differences only for the more volatile substances that are increasingly vented by prolonged gas flow. We in fact observed the corresponding reductions in the first part of the chromatograms shown in Figure 3. After some nearly constant peak heights in the central region (e.g. peaks at 14 and 19 min in Figure 3.), however, sharp decreases followed for which, again, we ignore the exact reason. Loss of peak height because of peak broadening is to be excluded since this effect would have primarily affected the earlier peaks. A saturation effect as it is linked with frontal analysis is excluded as well. We have some evidence that the losses are caused by displacement under the influence of the water and ethanol stream.

The inner surface of the glass capillary columns used had been modified by a series of purely inorganic treatments. This modification is very laborious and hard to reproduce. Its description would, therefore, be premature. The liquid phase (except for the inlet capillary) was Emulphor 0 with very slight alkaline reaction.

Auto exhaust gas

It seems to us that up to present the gasoline constituents being present in auto exhaust gases have been investigated (11-14) with different methods and quite separately from the organic exhaust components formed only in the hot motor (15,16). This caused us to try analyze simultaneously both groups of exhaust components.

Exhaust gases are saturated with water vapor. Therefore, the gas temperature must be equal or lower than that of the inlet capillary. Formation — and migration — of droplets is observed in a capillary cooled approx. 5° below the temperature of the gas sample.

Sampling was done very simply by quickly filling a 2 l round flask without markedly warming the glass walls. The flask was covered with aluminum foil through which the inlet capillary was introduced. As we were afraid of secondary reactions in the gas sample

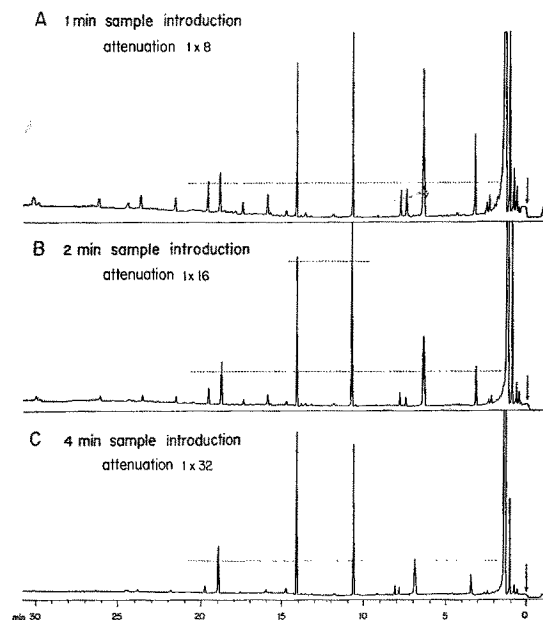


Figure 3. Cognac aroma head space. Different volumes of head space gas introduced into 1 m inlet capillary. Chromatographic conditions as for Figure 2. Atten. proportional to sample size.

we wished to reduce the time between sampling and introducing the capillary to a minimum. We obtained, however, similar chromatograms regardless of whether this time was 30 seconds or 3 hours.

To enhance bypassing of water vapor the inlet capillaries were coated with silicone oil SF 96. The main column was of the same type as that mentioned in the preceding section. The liquid phase was Emulphor 0 adjusted to slightly acidic reaction as needed because of the presence of phenols. For comparison purposes we used a second column coated with silicone oil SF 96. Identifications as listed in Table II are to be considered as tentative. They were done by adding pure substances to the sample and comparing the effects on both columns.

Chromatograms A and B in Figure 4 show results produced under identical sampling and chromatographic conditions from two motors consuming the same fuel. The gas samples were taken from the motor at low speed a few seconds after the cars had arrived from the same 5 mile drive. Chromatogram C, again

11. McEven, D. J., *Anal. Chem.* **38**, 1047 (1966).
12. Jacobs, E. S., *Anal. Chem.* **38**, 43 (1966).
13. Seizinger, D. E., *Instr. News, The Perkin-Elmer Co.* **18**, 11 (1967).
14. Maynard, J. B., and Sanders, W. N., *J. of the Air Pollution Control Assoc.* **19**, 505 (1969).
15. Barber, E. D., Sawicki, E., and McPherson, S. P., *Anal. Chem.* **36**, 2442 (1964).
16. Reckner, L. R., Scott, W. E., and Biller, W. F., *Proc. 30th Meeting American Petroleum Inst. Division Refining, Montreal, 1965.*

Table II. Substances identified in auto exhaust gas.

1 Benzene	13 1,2,3-
2 Toluene	Trimethylbenzene
3 Ethylbenzene	14 Indane
4 p-Xylene	15 1,2,4,5-
5 m-Xylene	Tetramethylbenzene
6 o-Xylene	16 Indene
7 1-Methyl-3-ethylbenzene	17 Acetophenon
8 Styrene	18 Naphthalene
9 1,3,5-Trimethylbenzene	19 2-Methylnaphthalene
10 1-Methyl-4-ethylbenzene	20 1-Methylnaphthalene
11 1-Methyl-2-ethylbenzene	21 Diphenyl
12 1,2,4-Trimethylbenzene	22 Phenol
	23 o-Cresol
	24 1,6-Dimethylnaphthalene
	25 p-Cresol
	26 m-Cresol

produced under the same chromatographic conditions, has been added to visualize the fundamental difference between fuel and exhaust vapors. It is seen that there is little difficulty to resolve both groups of exhaust components, those deriving from the fuel and those formed by cracking and partial oxidation, in the same run. The first significant member of the latter group is indene (peak 16) that is not present in gasoline. Whereas the naphthalenes are, in different concentrations, present in both fuel and exhaust the phenols clearly are not. The large peak reproducibly given off only by motor b and eluted at 48 min behaves chromatographically like a polycyclic aromatic hydrocarbon. Acenaphthene, acenaphthylene, and fluorene are eluted in this section but do not coincide with the mentioned peak.

The polar nature of the cracking and combustion products is clearly demonstrated by the comparison between chromatograms B/C in Figure 4 with A/B in Figure 5. Whereas from the polar columns they emerge well after the fuel substances, both groups remain mixed on the nonpolar column.

Conclusions

From the presented trace analytical applications we deduce the supposition that many actual analytical problems could be solved by similar methods using capillary columns. We again wish to emphasize, however, that, not knowing the special needs and difficulties in the individual working fields, we are not able to judge the actual applicability of our methods.

We are presently trying to modify for our columns Janak's (7) frontal analysis method with the aim of further increasing the concentration of trace substances from liquid as well as from gaseous mixtures.

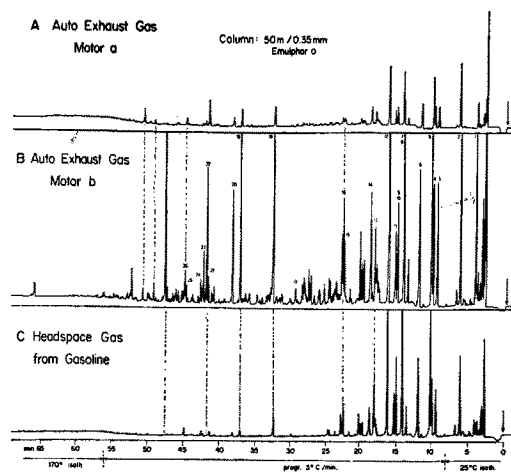


Figure 4. A and B: 2 ml of auto exhaust gas; C: 2 ml of gasoline head space gas. Glass capillary, 50 m/0.35 mm, Emulphor acidic. Carrier hydrogen, .6 atmos. (2.5 ml/min). App.: Carlo Erba, Mod. GI, FID, Atten. 1 x 16.

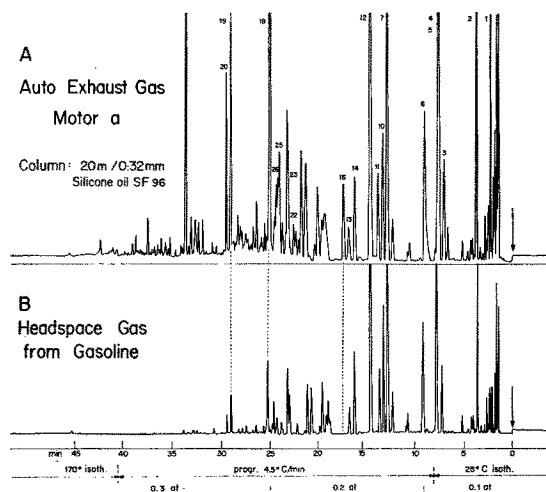


Figure 5. A: 2 ml of auto exhaust gas; B: 2 ml of gasoline head space gas. Glass capillary, 20 m/0.32 mm, silicone oil SF 96. Carrier hydrogen, .3 atmos. (2.0 ml/min). App.: Carlo Erba, Mod. GI, FID, Atten. 1 x 8.

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